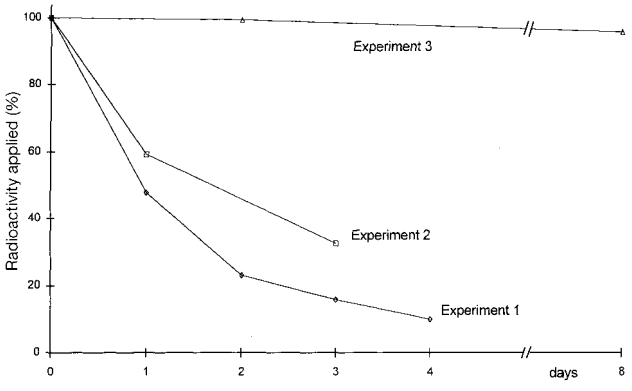

Figure 4. Photolysis of imidacloprid with incubation time. (◇) Experiment 1; DT_{50}; 0.7 days; radiation measured at leaves normalised to global radiation; 1020 J cm^{-2}; sum of global radiation measured at weather station; 6838 J cm^{-2}. (□) Experiment 2; DT_{50}; 1.4 days; radiation at leaves normalised to global radiation: 121 J cm^{-2}; sum of global radiation measured at weather station; 1460 J cm^{-2}. (△) Experiment 3 (in the dark).

proceeds via two routes. The first involves oxidation of the imidazoline ring, leading to the metabolite BNF 5540B, and the second involves a stepwise loss of the nitroimino group to give metabolite DIJ 9817.

The degradation rate depended very much on the amount of light falling on the leaf. There was very little degradation in Experiment 3 which was conducted in the dark (5% within eight days). By contrast, the time taken for 50% degradation of the applied compound (DT_{50}) under natural solar radiation was 0.7 and 1.4 days, respectively, in Experiments 1 and 2 (1.5th and second order, respectively). While the greater incident radiation in Experiment 1 led to a shorter DT_{50} value than for experiment 2, results for the latter experiment do not indicate a direct correlation between mean incident solar radiation (0.3–3.0 μm) and degradation. The degradation rates in the latter experiment were similar on day 1 and on days 2–3, even though the incident radiation on days 2–3 was 0.366 kJ cm^{-2} compared with 1.094 kJ cm^{-2} on day 1.

Conclusion

The photodegradation experiments reported here showed the presence of four compounds well-known as imidacloprid metabolites in plants. However, the identity of a new photometabolite, amounting to $\leqslant$3.8% of the applied radioactivity, was not established. Under field conditions, imidacloprid on tomato leaf surfaces is rapidly degraded, even under low light intensity conditions and it can be expected that degradation rates would be greater with greater exposure to light (greater angle of incidence of the sun, low latitudes, longer periods of daylight). The degradation of imidacloprid on leaf surfaces appears to be more complex than just photodegradation, although global radiation, presumably at certain specific wavelengths, plays a very important role.

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Selective mechanism of action of tebufenozide on lepidopteran cell lines

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Abstract: The non-steroidal ecdysone agonist, tebufenozide (RH-5992), induces a precocious incomplete molt primarily on lepidopteran insects, but has little or no effect on insects of other orders. 20 Hydroxyecdysone at 10^{-7} M induced the transcription factor CHR3 mRNA in CF-203 cells and DHR3 mRNA in DM-2 cells. Tebufenozide even at 10^{-10} M induced CHR3 mRNA in lepidopteran CF-203 cells, but even at 10^{-5} M it induced only trace levels of DHR3 mRNA in dipteran DM-2 cells. Studies using radiolabelled RH-5992 revealed that lepidopteran cell lines (CF-203 and MD-66) retained more of this compound within the cells than dipteran cell lines (DM-2 and K_c). The efflux of radiolabelled RH-5992 from DM-2 cells was temperature-dependent and was blocked by 10^{-5} M ouabain, an inhibitor of Na^+, K^+-ATPase, suggesting that the efflux was due to active transport.

Keywords: 20-hydroxyecdysone; tebufenozide; *Choristoneura* hormone receptor 3 (CHR3); *Drosophila* hormone receptor 3 (DHR3); ouabain

In the early 1980s, 1,2-diacyl-1-substituted hydrazines were found to induce precocious lethal molts in larvae. Among these, RH-5849 was the first compound that was shown to mimic the action of 20-hydroxyecdysone (20E) by acting through the ecdysteroid receptor.[1,2] Subsequently, tebufenozide (RH-5992), an analogue of RH-5849, was found to be very effective in inducing precocious lethal molts in lepidopteran larvae but had little or no effect on insects belonging to other orders. Two lepidopteran cell lines, FPMI-CF-203 (CF-203) and IPRI-MD-66 (MD-66) and two dipteran cell lines, DM-2 and K_c, were used to investigate the basis of lepidopteran specificity of tebufenozide *in vitro*. The mRNAs for hormone receptor 3 homologues (HR3) cloned from *Drosophila melanogaster* Meig (DHR3)[3] and *Choristoneura fumiferana* Clem (CHR3)[4] are expressed when ecdysteroid concentration rises during molts and are induced by treatment with 20E. Studies in several insects showed that HR3 mRNAs serve as

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suitable markers for studying ecdysone action.[3–5] Both lepidopteran (CF-203) and dipteran (DM-2) cell lines responded to 10^{-6} M 20E treatment, resulting in the induction of the HR3 mRNAs. Tebufenozide, on the other hand, was more effective in inducing detectable levels of CHR3 mRNA in CF-203 cells at concentration as low as 10^{-10} M than in inducing DHR3 mRNA in DM-2 cells even at levels as high as 10^{-5} M.

Studies on the relative uptake of [^{14}C]RH-5992 in the cell lines revealed that CF-203 cells accumulated six-fold more of [^{14}C]RH-5992 than DM-2 cells. The results were confirmed using a different lepidopteran (MD-66) and a dipteran (K_c) cell line. Since 20E evoked a similar response in both the lepidopteran and dipteran cell lines for the induction of HR3 mRNAs, we wondered if accumulation of ecdysteroids would be different in lepidopteran than in dipteran cell lines. In the absence of radiolabelled 20E, we used [^{3}H]Ponasterone A. Ponasterone A, like 20E, induced HR3 mRNA in the CF-203 and DM-2 cell lines at 10^{-6} M. [^{3}H]Ponasterone A accumulated at similar levels in the two cell lines during the first six hours, after which accumulation in the dipteran cell lines was greater. Thus, dipteran cell lines which were refractive to tebufenozide were found to be retaining less [^{14}C]RH-5992 than lepidopteran cell lines which responded to this compound. Similar results were also observed in one hymenopteran cell line (NL-28; Sundaram M Sohi SS and Palli SR, unpublished) and one coleopteran cell line (LD1-4; Dhadialla TS, unpublished).

Data from several experiments suggested that the lower accumulation of [^{14}C]RH-5992 in dipteran cells is due to active exclusion of this compound. First, when heat-killed (65 °C, 30 min) CF-203 and DM-2 cells were treated with [^{14}C]RH-5992, the accumulation of RH-5992 in the two cell lines was similar. Second, when DM-2 cells were incubated with [^{14}C]RH-5992 at 4 °C and 24 °C, there was 3.5-fold greater accumulation at the lower temperature. Therefore, it appears that [^{14}C]RH-5992 enters the cells (influx) passively. The CF-203 cells retain most of it, whereas DM-2 cells actively exclude (efflux) it. The active exclusion of [^{14}C]RH-5992 from the DM-2 cells seems to be an ATP-dependent process as revealed by the 3–4 fold increase in the [^{14}C]RH-5992 inside the DM-2 cells in the presence of ouabain, an inhibitor of Na^+,K^+ ATPase, which regulates the Na^+,K^+ pump.

An ATP-dependent cassette transporter which actively excludes steroid hormones, known as ligand effect modulator (LEM-1), has been characterized in yeast.[6] Investigations on multidrug resistance (mdr) in mammalian cells have shown that over-expression of mdr1 gene results in the over-production of a membrane protein, P-glycoprotein, which confers resistance to the cells by evoking an energy-dependent efflux of drugs resulting in decreased accumulation of drugs within the cells. Such an mdr-like gene has been isolated and characterized from *D melanogaster*.[7] The *D. melanogaster* cells used in this study may also have an mdr-like gene that may be involved in the efflux of RH-5992, resulting in reduced accumulation of [^{14}C]RH-5992 in DM-2 cells as compared to CF-203 cells.

It appears that there are at least two factors responsible for the lepidopteran specificity of tebufenozide. First, this analogue has a low binding affinity to the *Drosophila* ecdysteroid receptor complex[8] and second, it is actively excluded from the non-lepidopteran complex. There may be other unidentified factors that contribute to the lack of toxicity of tebufenozide to non-lepidopteran pests.

Further work on the influx/efflux mechanisms of tebufenozide in Coleopteran, Orthopteran and Hymenopteran cell lines is necessary to verify whether or not the mechanism of active exclusion is a general phenomenon in insects responsible for such refractiveness. Further studies to verify this hypothesis and to characterize the gene responsible for the active efflux mechanism in DM-2 cells are in progress.

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